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Original contribution

Heterogeneous mutational profile and prognosis conferred by *TP53* mutations in appendiceal mucinous neoplasms☆,☆☆

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Summary The eighth edition of American Joint Committee on Cancer (AJCC) advocates a 3-tier grading system for appendiceal mucinous tumors. The mutational profile for each tumor grade and the impact of *TP53* mutation on survival are unknown. We classified appendiceal mucinous tumors into 3 grades based on the eighth edition of American Joint Committee on Cancer: 21 G1 low-grade mucinous neoplasms, 21 G2 appendiceal adenocarcinomas, and 26 G3 signet ring cell carcinomas. Mutation profiles were obtained using next-generation sequencing. The impact of *TP53* on prognosis was investigated by multivariable analysis. Most G1 tumors harbor *KRAS/GNAS* mutations with *TP53* and *SMAD4* in a small subset of cases. G2 and G3 tumors show a more complex mutation pattern carrying *PIK3CA*, *BRAF*, or *TP53* mutations in addition to *KRAS/GNAS*. *PTEN* mutations were detected exclusively in G2 tumors. The prevalence of *KRAS* and *GNAS* mutations is significantly lower in G3 tumors relative to G1/G2, whereas *TP53*, *PIK3CA*, or *BRAF* mutations are common. Mutations in *NRAS*, *IDH2*, *CDH1*, *RB1*, *CTNNB1*, *CDKN2A*, *PTPN11,* and *KIT* genes were observed in single cases. Patients with *TP53*-mutated disseminated G2 and G3 tumors had worse progression-free survival than did those with wild-type *TP53* tumors ($P = .0315$). A trend toward worse overall survival was observed in *TP53*-mutated G3 tumors ($P = .102$). p53 expression correlated with mutation status. We demonstrate a distinct but overlapping pattern of gene mutations in each

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grade of appendiceal mucinous tumors and the independent impact of *TP53* mutation on progression-free survival but not overall survival.

1. Introduction

Appendiceal malignancies are rare, with an incidence of 0.12 cases per 1 000 000 people per year [1]. Patients with appendiceal tumors often present with acute appendicitis, or pelvic/abdominal masses [1]. Among the epithelial neoplasms of the appendix, mucinous tumor is the most frequent diagnosis [2]. These tumors characteristically cause dilation of appendiceal lumen with accumulation of gelatinous mucin and may spread into peritoneum producing a pseudomyxoma peritonei (PMP) [3]. The fourth edition of World Health Organization (WHO) *Classification of Tumours of Digestive System* classifies these mucinous tumors either as low grade, which encompasses low-grade appendiceal mucinous neoplasm (LAMN) and low-grade PMP, or as high grade, which includes mucinous carcinoma and high-grade PMP [4]. More recently, the eighth American Joint Committee on Cancer (AJCC) edition adopted a 3-tier morphologic grading system, which is incorporated into prognostic staging. The histopathologic characteristics of 3 subgroups were described by Davison et al [5], who showed that clinical outcome is strongly correlated with tumor grade.

Genetic alterations of appendiceal mucinous tumors are not identical to their counterpart colorectal carcinoma [6]. Gene mutations in APC and β-catenin are common in colorectal carcinoma but are rare in appendiceal mucinous tumors [6,7]. Microsatellite instability, defined by DNA mismatch repair deficiency, is seldom found in appendiceal mucinous carcinomas or PMP [8]. Recent molecular studies have investigated the genetic alterations in appendiceal neoplasms. Loss of chromosome 18q and *SMAD4* (*DPC4*) mutations are detected in a subset of appendiceal adenocarcinoma [7]. Subsequent genomic profiling of mutations in mucinous appendiceal tumors by next-generation sequencing (NGS) suggested that the mutation profile may be different between low- and high-grade morphologic subtypes [6,9-11]. *KRAS* and *GNAS* are the most frequently identified mutations. Rare mutations such as *STK11*, *NRAS*, and *MET* are found in single cases [9]. However, previous studies focused either on high-grade and low-grade PMP [10,11] or on LAMN and adenocarcinoma of the appendix [9]. With the adoption of the new 3-tier grading scheme recommended by AJCC, the comparison of mutation profiles in appendiceal mucinous tumors categorized as grade 2 adenocarcinoma and grade 3 signet ring cell carcinoma (SRC) has not been well investigated.

TP53 mutations are often seen in high-grade appendiceal tumors. *TP53* mutations are associated with poor prognosis in gastric and esophageal carcinoma, but the role of these mutations is inconclusive in other tumor types [12-14]. To date, 2 studies have investigated the relationship of aberrant p53 immunoreactivity with clinical outcome in appendiceal tumors [11,15]. In one study, abnormal *TP53* expression was an independent predictor of overall survival in PMP. However, the other study found no statistical significance regarding aberrant p53 immunostaining status when PMP is stratified into low-grade and high-grade tumors. Thus, the prognostic significance of p53 aberration in appendiceal tumors remains inconclusive and has not been studied at the DNA mutation level.

We performed NGS on appendiceal mucinous tumors from 68 patients, the largest number of cases reported to date, and characterized the mutational profiles according to tumor grade specified by AJCC eighth edition recommendations.

The role of *TP53* mutation in progression-free survival and overall outcome is also investigated by multivariate study controlling for age, sex, tumor grade, lymph node involvement, staging, surgery, HIPEC, and systemic chemotherapy.

2. Materials and methods

2.1. Cases

The study population consists of 68 patients with appendiceal mucinous tumors resected at the University of Massachusetts Memorial Medical Center between 2004 and 2016.

To assign a diagnosis based on the 3 histomorphologic grades advocated by the AJCC eighth edition, all tumors were reviewed by 2 gastrointestinal pathologists. The categories are enumerated below (Fig. 1):

(1) Grade 1 (G1; n = 21): LAMN composed of copious mucin pools with scattered stripes of mucinous epithelium displaying a single layer of basically located small round and regular nuclei.
(2) Grade 2 (G2; n = 21): mucinous adenocarcinoma composed of small mucin pools with neoplastic mucinous epithelium demonstrating destructive invasive and high-grade cytologic grade (nuclear enlargement, stratification and crowding, prominent nucleoli, and vesicular chromatin).
(3) Grade 3 (G3; n = 26): high-grade mucinous adenocarcinoma consisted of greater than 50% of signet ring cells.

Clinical history and treatment information were obtained and reviewed with the approval of the institutional review board.

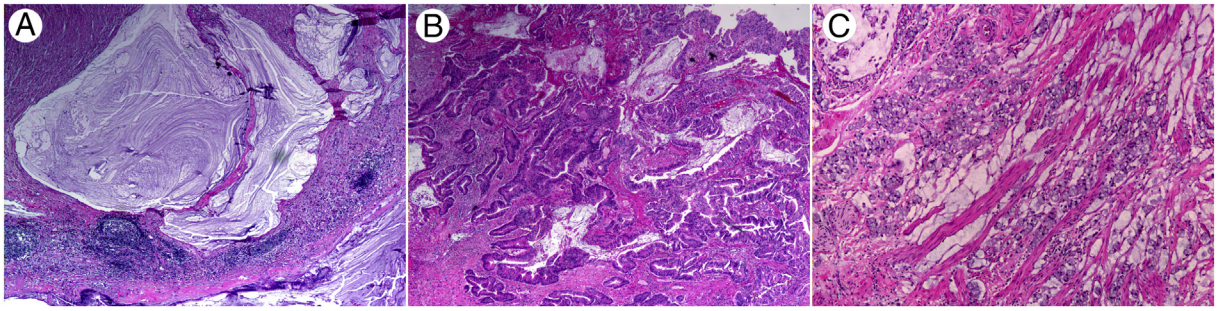

Fig. 1 Histomorphology of appendiceal mucinous tumors (hematoxylin and eosin stain). A, Disseminated LAMN (grade 1; magnification ×100) with copious mucin pools and scattered stripes of epithelium displaying mild cytologic atypia. B, High-grade mucinous adenocarcinoma (grade 2; magnification ×200) with destructive invasion and predominant small mucin pools. The neoplastic epithelium demonstrated high cytologic grade. C, High-grade mucinous adenocarcinoma with signet ring cells (grade 3; magnification ×200).

2.2. Next-generation sequencing

DNA was extracted from formalin-fixed, paraffin-embedded tissue (Qiagen, Germantown, MD) and analyzed by NGS for "hotspot" mutations in 50 cancer genes (Ampliseq Cancer Hotspot Panel v2; Thermofisher Scientific, Waltham, MA) as described previously [16,17].

2.3. Immunohistochemistry

p53 immunohistochemistry (IHC) analysis was performed on specimens with sufficient tissue (4 G1, 10 G2, and 22 G3 tumors). Five-micron formalin-fixed, paraffin-embedded tissue sections were stained using a monoclonal antibody cocktail of 2 clones (Ab2 and Ab6; Calbiochem, San Diego, CA) as previously described [18]. A positive p53 IHC result was defined as positive staining in the nucleus of tumor cells.

2.4. Statistical analysis

The correlations between *TP53* mutation and clinicopathological factors including age, sex, grade of tumor, and lymph node involvement were analyzed using the Fisher exact test and the χ^2 test. Overall survival was identified as the time (measured in months) from initial diagnosis to death and censored at last clinical follow-up. Time to disease progression was defined as time (measured in months) from initial therapy to the first documented clinical evidence of progression (ie, relapse or metastasis) and censored at the time of last clinical contact or death. Multivariable overall survival and progression-free survival were performed using the Kaplan-Meier method and the Cox proportional hazards model and considered the clinicopathological factors above plus surgery, comorbidities, HIPEC, and systemic chemotherapy. Because of the limited number of patients with pT1, pT2, or pT3 disease, the analysis was limited to pT4 disease, eliminating any effect of pT stage on outcome or progression. All *P* values with levels <.05 are considered statistically significant. Statistical analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Mutational profiles

Most tumors (18/21) in G1 category exhibited 1 or 2 mutations. Eleven cases had a double *KRAS*/*GNAS* mutation, and 5 cases harbored a single *KRAS* mutation. One case contained a single *NRAS* mutation. The remaining 4 cases carried extra mutation(s) in addition to *KRAS* and/or *GNAS* mutation(s) (Table 1). In total, *KRAS* and *GNAS* mutations were seen in 19 (90%) and 13 (62%) of 21 grade 1 cases, respectively (Fig. 2). The other mutations detected were *TP53* (n = 2; 10%), *SMAD4* (n = 2; 10%), IDH2 (n = 1), *NRAS* (n = 1), and CDKN2A (n = 1).

G2 tumors demonstrated more complex mutation patterns, with 9 cases of 21 showing at least 3 mutations. Except for 6 cases with the double *KRAS*/*GNAS* mutation and 3 cases with a single *KRAS* mutation, the other 12 tumors possessed various mutation combinations (Table 1). Similar to G1 tumors, *KRAS* and *GNAS* mutations were identified in 18 (86%) and 14 (67%) of G2 cases, respectively. By contrast, *TP53* (n = 6; 29%) and *SMAD4* (n = 4; 19%) mutations were found more frequently in grade 2 than in grade 1 tumors (Fig. 2). In addition, some G2 tumors acquired *PIK3CA* (n = 4; 19%) and *BRAF* (n = 2; 10%) mutations, which were also present in grade 3 tumors (Fig. 1). The remaining low-frequency mutations found exclusively in G2 tumors are listed in Table 1.

Among the 26 G3 tumors, a double *KRAS*/*GNAS* mutation and single *KRAS* mutation were seen only in 2 and 5 cases, respectively. No mutations were detected in 7 cases. All remaining cases showed different combinations of 1, 2, 3, or 4 mutations (Table 1). The frequency of *KRAS* (n = 12; 46%) and *GNAS* (n = 3; 12%) mutations was significantly reduced in G3 (Fig. 2) relative to G1 (*KRAS*, *P* = .0014; *GNAS*, *P* = .00029) and G2 tumors (*KRAS*, *P* = .005; *GNAS*, *P* = .0038).

Table 1 Composition of genetic mutations in each grade of disseminated appendiceal mucinous tumors

Grade 1		Grade 2		Grade 3	
Mutation profiles	No. of cases (%)	Mutation profiles	No. of cases (%)	Mutation profiles	No. of cases (%)
KRAS/GNAS	11 (52)	*KRAS/GNAS*	6 (29)	*KRAS/GNAS*	2 (8)
KRAS	5 (24)	*KRAS*	3 (14)	*KRAS*	4 (15)
KRAS/GNAS/SMAD4/TP53	1 (5)	*KRAS/GNAS/TP53*	2 (10)	*KRAS/GNAS/TP53/SMAD4*	1 (4)
KRAS/IDH2	1 (5)	*KRAS/GNAS/SMAD4*	1 (5)	*TP53/SMAD4*	1 (4)
NRAS	1 (5)	*KRAS/GNAS/PIK3CA*	1 (5)	*TP53/KRAS*	1 (4)
GNAS/SMAD4/TP53	1 (5)	*KRAS/SMAD4*	1 (5)	*TP53/KRAS/PIK3CA*	1 (4)
KRAS/CDKN2A	1 (5)	*SMAD4/RB1*	1 (5)	*TP53*	1 (4)
		GNAS/SMAD4/TP53/CTNNB1/PTPN11	1 (5)	*SMAD4*	1 (4)
		KRAS/GNAS/KDR/PIK3CA	1 (5)	*BRAF*	1 (4)
		KRAS/GNAS/KDR/PIK3CA/KIT	1 (5)	*BRAF/CDKN2A*	1 (4)
		KRAS/TP53/PTEN	1 (5)	*KRAS/SMAD4/TP53*	1 (4)
		BRAF/PTEN/TP53	1 (5)	*KRAS/APC/TP53*	1 (4)
		KRAS/GNAS/SMAD4/TP53/PIK3CA	1 (5)	*KRAS/MET*	1 (4)
				CDH1/PIK3CA	1 (4)
				ABL	1 (4)
				ND	7 (27)
Total no. of cases	21		21		26

Abbreviation: ND, not detected.

SMAD4 and *TP53* mutations were found in 3 (12%) and 7 (27%) of grade 3 cases, respectively. *PIK3CA* and *BRAF* were shown in 2 (8%) cases each.

3.2. Patient demographics

The study cohort, comprising 21 cases of G1 LAMN, 21 cases of G2 ACA, and 23 cases G3 SRC, had a median age of 49.7, 56.7, and 57.7 years, respectively (Table 2). Three of 26 cases of G3 SRC were omitted from analysis because the clinical information was not accessible. G1 and G2 cases showed a slight female predominance of 1.6–1.8:1. Fourteen (67%) of 21 G1 tumors, all 21 G2 tumors (100%), and 19 (91%) of 21 G3 tumors are staged as pT4. All the pT4 tumors

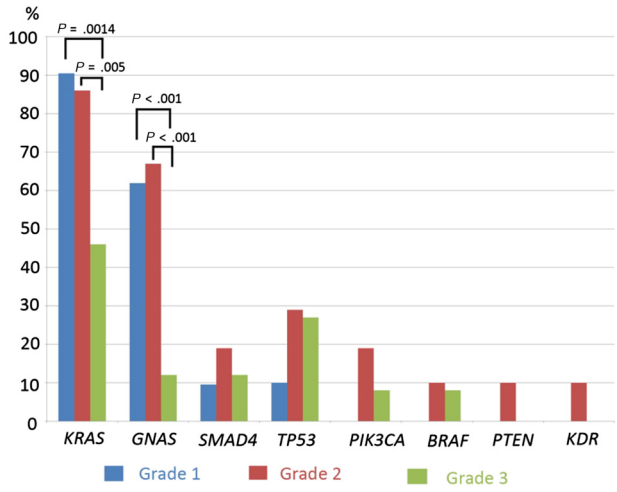

Fig. 2 Genes mutated in more than one sample. The frequency of *KRAS* and *GNAS* mutations in G3 SRC significantly declined compared with those in G1/G2 tumors. The prevalence of *TP53* mutations is higher in G2/G3 tumors (29% and 27%, respectively) than in G1 tumors (10%). *PIK3CA* and *BRAF* mutations are identified in G2/G3 tumors but not in G1 tumors. PTEN and KDR variants are only detected in G2 tumors.

Table 2 Patient demographics

Variables	AJCC tumor grade		
	G1	G2	G3
Age at diagnosis (y), mean (SD)	49.7 (12.0)	56.7 (11.0)	57.7 (12.3)
Sex (%)			
M	8 (38.1)	12 (57.1)	8 (34.8)
F	13 (61.9)	9 (42.9)	15 (65.2)
Pathologic stage (%)			
pT2	1 (4.8)	0 (0)	0 (0)
pT3	6 (28.6)	0 (0)	2 (8.7)
pT4	14 (66.7)	21 (100)	21 (91.3)
Lymph node involvement (%)			
Yes	0	1 (4.8)	16 (69.6)
No	0	5 (23.8)	4 (17.4)
N/A	21 (100)	15 (71.4)	3 (13.0)
HIPEC (%)			
Yes	11 (52.4)	10 (47.6)	11 (47.8)
No	10 (47.6)	11 (52.4)	12 (52.2)
Systemic chemotherapy (%)			
Yes	4 (19.0)	20 (95.2)	22 (95.7)
No	17 (81.0)	1 (4.8)	1 (4.3)
TP53 mutation (%)			
Yes	2 (9.5)	6 (28.6)	7 (26.9)
No	19 (90.5)	15 (71.4)	19 (73.1)

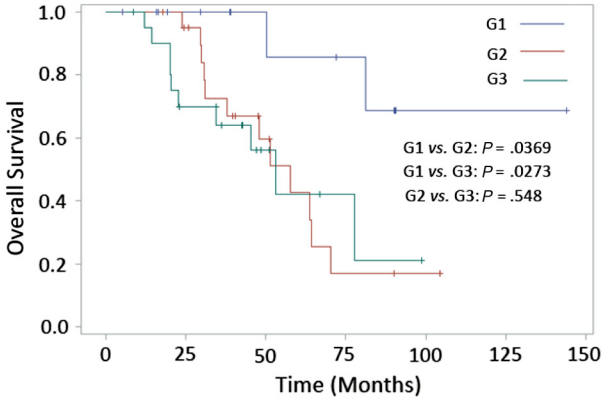

Fig. 3 Kaplan-Meier curve for overall survival among patients with AJCC G1, G2, or G3 disseminated stage IV appendiceal mucinous tumors. Patients with G1 tumors have significantly better overall survival compared with those with G2/G3 tumors (G1 versus G2, $P = .0369$; G1 versus G3, $P = .0273$). There is no significant difference in overall survival for patients with G2 or G3 tumors ($P = .548$).

were also clinical stage IV with peritoneal dissemination. All patients underwent surgical tumor resection. One (4.8%) G2 tumor and 16 (69.6%) G3 tumors had lymph node metastasis. Eleven (52.4%) patients with G1 tumors, 10 patients (47.6%) with G2 tumors, and 11 (47.8%) patients with G3 tumors underwent 1 episode or multiple episodes of HIPEC chemotherapy. Four (19.0%) patients with G1 tumors received systemic chemotherapy. Patients with G2 and G3 tumors received systemic chemotherapy, with the exception of 1 patient from each of these groups.

3.3. Overall disease-specific survival is greater in G1 than in G2/G3 tumors

Among 68 patients with clinical stage IV tumors, 56 were followed up clinically, including 14 of 21 patients with morphologic G1 tumors, 21 of 21 patients with G2 adenocarcinoma, and 21 of 26 patients with G3 SRC. The median follow-up interval was 40 months from initial diagnosis. Patients' age, sex, status of regional lymph node metastasis, comorbidities, and use of HIPEC or adjuvant chemotherapy were considered variables for risk stratification. Multivariable analysis showed that patients with G1 neoplasms had significantly longer survival relative to patients with G2 and G3 tumors ($P = .0369$ and $P = .0273$, respectively; Fig. 3). By comparison, patients with G2 and G3 tumors did not exhibit significant difference in overall survival ($P = .548$; Fig. 3).

3.4. Clinicopathological association of *TP53* mutation

TP53 mutation was detected in 15 (24.6%) of 65 patients with appendiceal mucinous tumors, including 2 G1, 6 G2, and 7 G3 cases. *TP53* mutation status was not significantly different between tumor grades (Table 3; G1 versus G2, $P = .238$; G1 versus G3, $P = .137$). *TP53* mutational status showed no significant association with any of these clinicopathological features (Table 3).

3.5. Prognostic significance of a *TP53* mutation

Because previous studies suggested that aberrant p53 protein may be associated with poor prognosis, we investigated whether *TP53* mutation impacts survival. Using Kaplan-Meier survival functions, a trend toward worse overall survival was observed in patients with *TP53*-mutated G3 tumors; however, no significant difference was identified in either G2 or G3 tumor category ($P = .328$ and $P = .102$, respectively; Fig. 4). A *TP53* mutation was a significant adverse risk factor for progression-free survival for patients with G2 tumors ($P = .0268$; Fig. 4). *TP53*-mutated G3 tumors also showed a trend toward higher risk of disease progression; however, the trend did not reach statistical significance ($P = .0756$; Fig. 4). Because no statistical significance of survival was found between G2 and G3 populations (Fig. 3), we combined G2 and G3 data. Progression-free survival was significantly inferior in the *TP53*-mutant group relative to the wild-type *TP53* group ($P = .0315$; Fig. 4). A trend toward reduced survival was observed in the *TP53*-mutated group ($P = .0692$).

Table 3 Clinicopathological association of *TP53* mutation

Patient data	Total (n = 65)	*TP53* mutation (n = 15)	*TP53* wild type (n = 50)	*P*
Age at diagnosis (y), mean (SD)	54.9 (12.0)	55 (12.0)	54.8 (12.1)	.958
Sex				1.000
Female (%)	37	9	28	
Male (%)	28	6	22	
Grade				
G1	21	2	19	.115 (G1 versus G2 + G3)
G2	21	6	15	.238 (G1 versus G2)
G3	23	7	16	.137 (G1 versus G3)
Lymph node involvement				
Present	17	6	11	1.000
Absent	9	2	7	

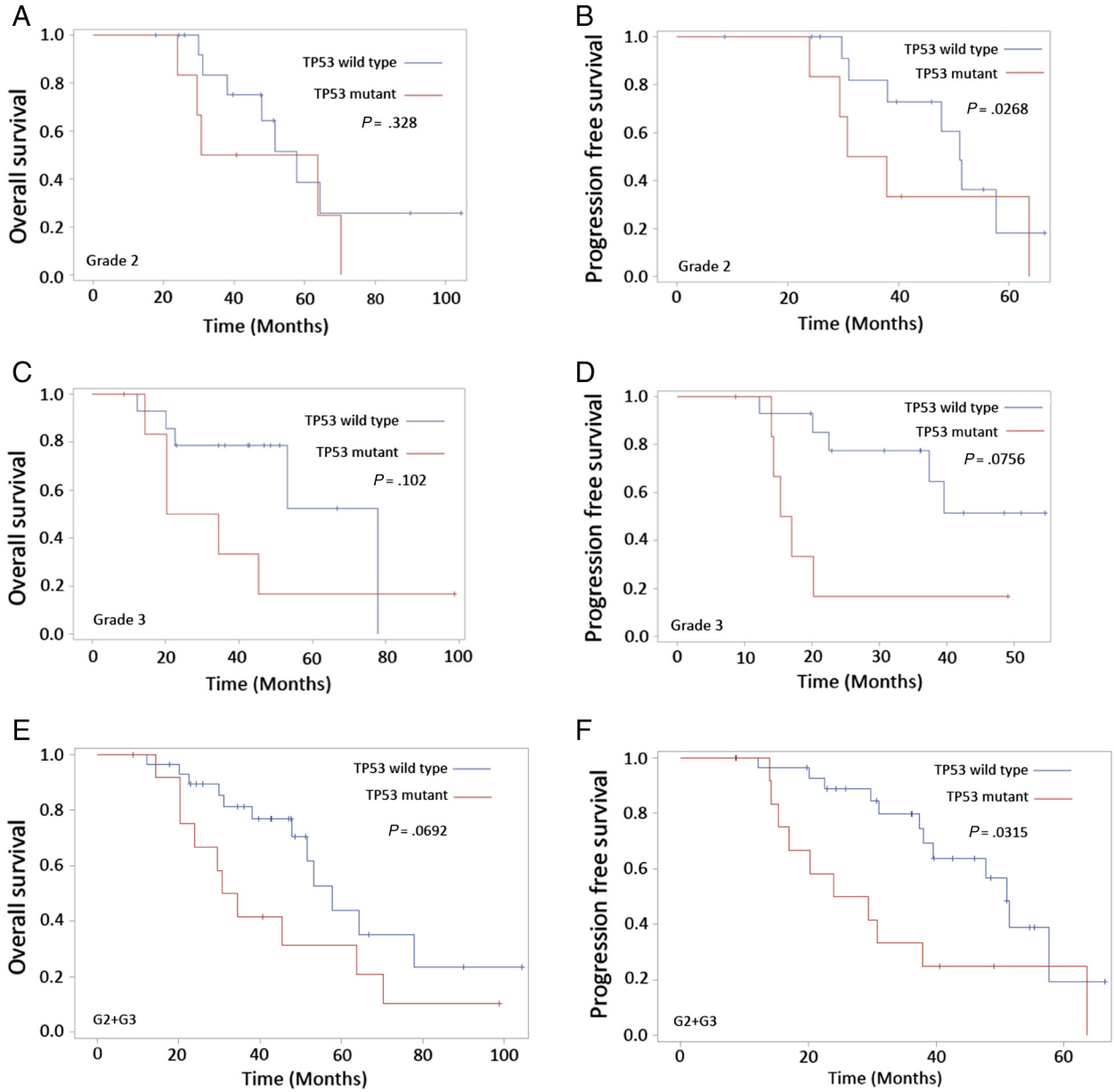

Fig. 4 Prognostic values of *TP53* mutation in patients with disseminated stage IV appendiceal mucinous tumors. Kaplan-Meier curves illustrate a trend toward inferior overall survival and progression-free survival of patients with *TP53*-mutated G2 (A and B), G3 (C and D), or combined G2/G3 tumors (E and F) compared with those with wild-type *TP53* tumors. There is a significant difference in progression-free survival for patients with or without *TP53* mutation in the G2 group and the G2 + G3 combined group ($P = .0268$ and $P = .0315$, respectively).

When G1, G2, and G3 tumors are combined for univariate analysis, overall survival and progression-free survival were significantly worse in the *TP53* mutant group than in the wild-type *TP53* group ($P = .0496$ and $P = .0174$, respectively). Using Cox proportional hazards multivariate modeling showed that only grade was an independent prognostic factor for both overall survival and progression-free survival (Table 4; $P = .0329$ and $P = .0053$, respectively). *TP53* mutational status ($P = .211$ and .0528), age ($P = .560$ and .358), sex ($P = .440$ and .413), lymph node involvement ($P = .669$ and .500), HIPEC therapy ($P = .0579$ and .491), or systemic chemotherapy ($P = .502$ and .256) was not significant in either overall survival or progression-free survival (Table 4).

3.6. p53 IHC correlates with *TP53* mutation

All *TP53* mutant and representative wild-type *TP53* cases with adequate tissue were stained for p53 protein. Eleven of 12 cases with a *TP53* substitution mutation showed strong and diffuse nuclear p53 expression in tumor cells (Fig. 5). One case with *TP53* nonsense (stop-codon) mutation had

Table 4 Multivariate analysis of overall survival and progression-free survival of disseminated stage 4 appendiceal mucinous adenocarcinoma according to histologic, molecular, and clinical features

Variable	Patients	HR (95% CI)	*P*	HR (95% CI)	*P*
		Overall survival		Progression-free survival	
Patient age (y)	56	1.01 (0.97-1.06)	.560	1.02 (0.98-1.07)	.358
Sex					
Female	32	Referent		Referent	
Male	24	1.44 (0.57-3.67)	.440	1.50 (0.57-3.95)	.413
AJCC grade					
G1	14	Referent		Referent	
G2/G3	21/21	2.32 (1.07-5.03)	.0329	3.30 (1.42-7.63)	.0054
Lymph node involvement					
Absent	9	Referent		Referent	
Present	17	1.73 (0.14-21.0)	.669	2.27 (0.21-24.8)	.500
HIPEC					
Given	31	Referent		Referent	
Not given	25	0.35 (0.12-1.04)	.0579	0.690 (0.24-1.98)	.491
Systemic chemotherapy					
Given	43	Referent		Referent	
Not given	13	1.71 (0.36-8.09)	.502	2.53 (0.51-12.55)	.256
Comorbidity					
Absent	50	Referent		Referent	
Present	6	1.26 (0.33-4.85)	.739	0.99 (0.25-3.95)	.983
TP53 mutation					
Negative	40	Referent		Referent	
Positive	16	1.90 (0.69-5.21)	.211	2.80 (0.99-7.95)	.0528

Abbreviations: CI, confidence interval; HR, hazard ratio.

p53-NULL immunophenotype. All wild-type *TP53* cases were negative for p53 staining.

4. Discussion

Our study represents the largest mutational analysis of disseminated appendiceal mucinous tumors by NGS to date, as well as the first study to investigate the mutational profile of these tumors categorized according to the AJCC eighth edition. We are also the first to investigate the prognostic impact of *TP53* mutations in patients with grade 2 appendiceal adenocarcinoma or grade 3 SRC.

Previous studies evaluating the genetic alterations in appendiceal mucinous tumors detected a heterogeneous mutation spectrum in low-grade or high-grade tumors specified by WHO fourth edition [6,9-11]. We observed a similar pattern using the new AJCC grading system, which separates high grade into G2 and G3 categories. G1 tumors harbor *KRAS*/*GNAS* mutations with other mutations in rare cases. In contrast, G2 and G3 tumors carry a more complex mutation spectrum. In addition, the prevalence of *KRAS*/*GNAS* mutations in G3 tumors is significantly lower than in G1/G2 tumors. This finding supports the separation of high grade into G2 and G3 categories. In previous studies, neither *KRAS* nor *GNAS* mutation exhibited any prognostic significance [19,20].

Zauber and colleagues [21] reported that all of low-grade mucinous tumors had *KRAS* mutation either in codon 12/13, with G12D and G12V being the most common. Similarly, we found *KRAS* mutations were most frequent in codon 12/13, involving p.G12 V, p.G12D, p.G13D, and p.G13S, and less frequently in codons 61/146. *KRAS* mutations contribute to colorectal carcinogenesis, and codon 12/13 mutations are associated with poor response to anti–epidermal growth factor receptor (anti-EGFR) therapies, such as cetuximab [22]. Because patients with appendiceal carcinoma receive the same systemic therapy regimen as colorectal cancer patients, *KRAS* status may impact management. Nevertheless, a large cohort study demonstrated that anti-EGFR therapy did not provide clinical benefit for patients with *KRAS* wild-type appendiceal tumors [19].

Our study found that mutations in other genes downstream of EGFR such as *BRAF* (eg, p.D594N and p.V600E) and *PIK3CA* were exclusively identified in G2 and G3 tumors, but not in G1 LAMN. Nummela et al [11] also reported a *BRAF* V600E mutation in 1 case of high-grade PMP. Unlike *BRAF* V600E, the *BRAF* p.D594N mutation impairs *BRAF* kinase activity and has been reported in other tumors [17,23]. MEK inhibitors or RAF inhibitors may benefit patients with concomitant kinase-silence *BRAF* mutation and activating RAS mutation [23]. In other studies, *PIK3CA* mutations were observed in 2 cases of adenocarcinoma with goblet cell features and a single case of low-grade PMP [9,11]. *PIK3CA* is

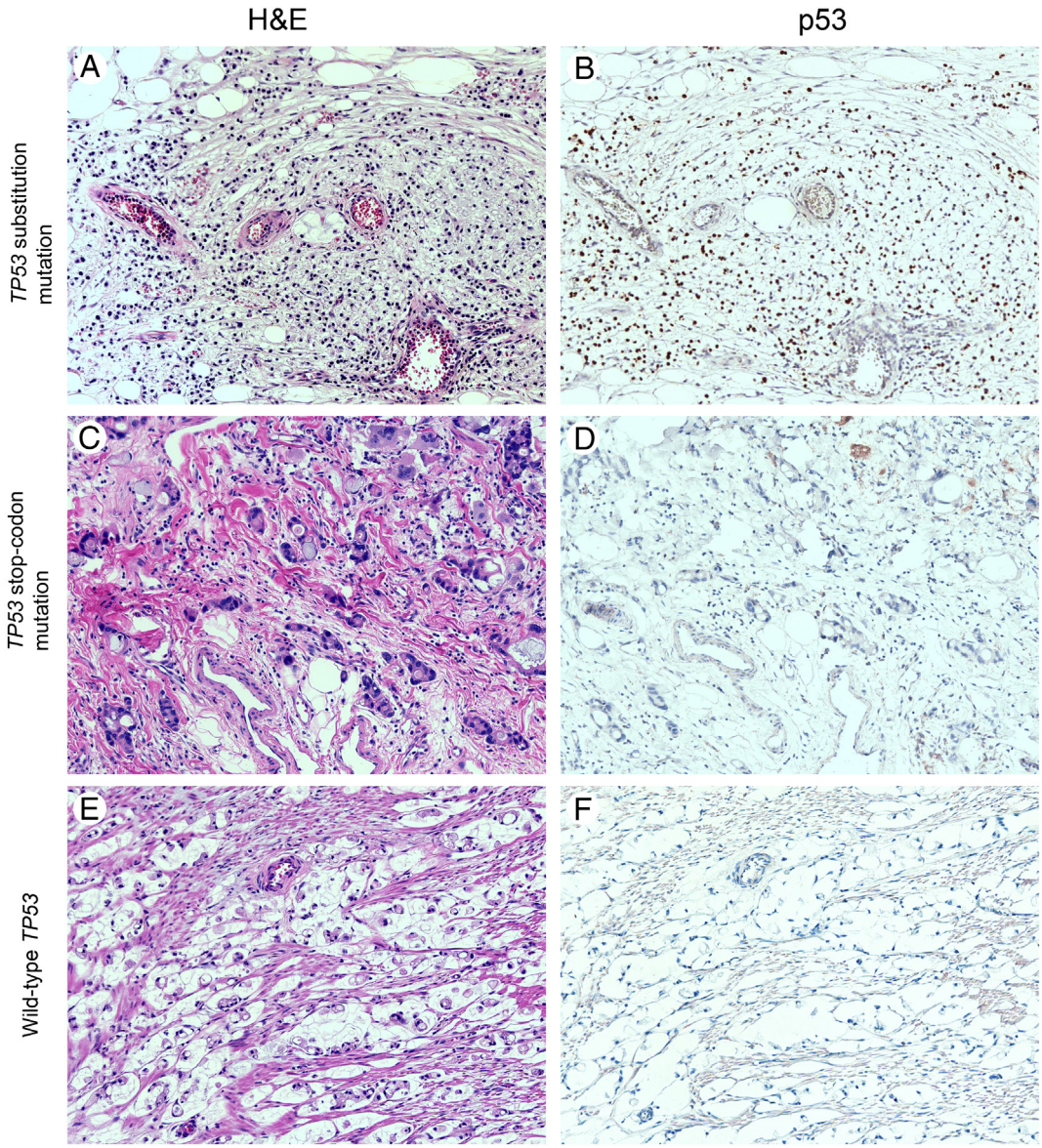

Fig. 5 p53 immunohistochemical stains in SRCs with mutant or wild-type *TP53* (magnification ×200). A and B, SRC with *TP53* substitution mutation resulting in strong and diffuse p53 expression in carcinoma. C and D, *TP53* nonsense (stop-codon) mutation with associated null phenotype of p53. E and F, SRC without *TP53* mutation with associated wild-type p53 immunostaining.

a target in ongoing clinical trials (eg, NCI Match trial [24]) and may have therapeutic implications for these patients in the future.

Activation of TGF-β pathway also contributes to the pathogenesis of appendiceal carcinoma. *SMAD4* mutation/deletion has been reported in both low-grade and high-grade PMP as well as adenocarcinoma with goblet cell features [6,9,11]. We identified *SMAD4* mutations in all 3 grades of appendiceal mucinous tumors with similar prevalence among each grade. *TP53* mutation was also identified in all 3 tumor grades but showed a relatively higher prevalence in G2 and G3 tumors versus G1 tumors ($P = .115$), consistent with previous studies based on the WHO classification [9,10].

Regarding the clinical outcome of patients with disseminated appendiceal tumors, multiple clinical variables should be taken into consideration [25,26]. Like previous studies, age, sex, HIPEC therapy, lymph node involvement, or systemic chemotherapy had no significant effect on patient survival in our cohort [5,20]. Consistent with previous studies, tumor grade acts as an independent prognostic variable and higher tumor grade predicts a worse clinical outcome [5,20,27]. Similar to Davison and colleagues [5], we found that patients with G1 tumors exhibited a better prognosis, but overall survival between patients with G2 and G3 tumors was not statistically different.

To eliminate the effect of tumor grade and isolate the impact of *TP53* on prognosis, we investigated patients with G2

or G3 stage IV tumors. Patients with a *TP53* mutation showed a trend toward worse progression-free and overall survival. A significant effect of *TP53* mutation was only observed in G2 tumors. In our study, most *TP53* mutant tumors showed p53 overexpression, consistent with stabilization of the *TP53* protein leading to aberrant p53 staining [28]. Less commonly tumors with a *TP53* stop codon or frameshift mutation yielded no p53 staining. This finding supports the use of p53 IHC as a surrogate for *TP53* mutation status, albeit with slightly lower sensitivity.

In univariable analysis, Shetty et al [15] found that p53 overexpression in PMP was associated with significant worse overall survival. However, further stratification of PMP into low- and high-grade indicated that p53 overexpression had no impact on survival. By contrast, Nummela and colleagues [11] showed that aberrant p53 staining was an independent predictor of inferior overall survival in a patient cohort with low- and high-grade tumors, whereas tumor grade was not. It is important to note that aberrant p53 staining has been found in a significantly higher proportion of high-grade than in low-grade tumors in both of these studies [11,15]. Together with our findings, this raises the possibility that tumor grade rather than *TP53* status has the greater impact on overall survival [15].

We used NGS profiling and p53 IHC to characterize *TP53* mutation status distinguishing this study from previous reports. Sequencing identified mutations that stabilize protein expression as well as a null phenotype providing greater sensitivity than IHC by itself. Despite this advantage, the role of *TP53* mutation in the prognosis of patients with disseminated appendiceal mucinous tumors remains inconclusive. A larger data set or meta-analysis is needed to establish the role of *TP53*.

In summary, AJCC G1 tumors harbor predominantly *KRAS* and *GNAS* mutations, while G2 and G3 tumors harbor more complex mutational profiles and tend to have greater number of gene mutations. *TP53* mutations are more frequently found in stage IV G2 and G3 tumors and are associated with worse progression-free survival, but did not reach significance for overall survival. Our results suggest that gene mutation analysis may identify patients at higher risk for progression and aid in selecting appropriate targeted therapies.

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